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Reproductive and Haemato-Biochemical influence of aqueous extracts of *Moringa oleifera* leaves on adult New Zealand Rabbit Bucks

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Abstract
The difficulty in filling the niche in the animal protein need of ever expanding populations of developing countries; and the advocacy for organic farming have diversified research into natural, unconventional and sustainable means to proffer solutions. This research lasted for four weeks and was carried out in the Rabbitry Unit of the Teaching and Research Farm, Federal University of Technology, Owerri, Imo State, Nigeria. Eighteen adult New Zealand rabbit bucks were grouped into three, containing six rabbits each; replicated three times to contain two rabbits per replicate. Three treatments; MT_0 (0.0 g *Moringa oleifera* leaves extracts per litre of distilled water), MT_{100} (100.0 g *Moringa oleifera* leaves extracts per litre of distilled water) and MT_{200} (200.0 g *Moringa oleifera* leaves extracts per litre of distilled water) were randomly assigned and orally administered to the three rabbit groups. Semen volume, percent abnormal sperm and reaction time of the experimental rabbits were significantly ($P<0.05$) lower in the MT_{100} and MT_{200} rabbits than in the MT_0 (control) rabbits, while total sperm count, sperm concentration, sperm motility, percent live sperm and libido score were significantly ($P<0.05$) higher in MT_{100} and MT_{200} rabbits than in MT_0 rabbits. The haematological parameters of the experimental rabbits recorded significantly ($P<0.05$) higher packed cell volume (PCV) and haemoglobin (Hb) concentration in MT_{200} than in MT_0 and MT_{100}, which were similar ($P>0.05$). Total white blood cell (WBC) count was similar ($P>0.05$) between MT_{100} and MT_{200}, which were significantly ($P<0.05$) higher than WBC count recorded for MT_0. Rabbits on MT_{100} and MT_{200} recorded similar ($P>0.05$) values in the serum total protein (TP) and globulin, which were significantly ($P<0.05$) higher than the value recorded for MT_0 rabbits. Serum urea and urea/creatinine ratio of the rabbits were similar ($P>0.05$) between MT_0 and MT_{100}, but significantly ($P<0.05$) higher in MT_{200}; while serum glucose and cholesterol recorded a dose dependent significant ($P<0.05$) inverse decrease in the experimental rabbits. From the results of this study, it can be concluded that aqueous extracts of *Moringa oleifera* leaves can be used in rabbit production to enhance immunity, serum protein synthesis and reproductive performance of New Zealand rabbit bucks.

Keywords: Immunity, libido, semen parameters, serum protein synthesis.

Introduction

The increasing populations of developing countries like Nigeria, and subsequent increase in animal protein need, has directed attention towards micro livestock species like rabbits [1]. Based on the numerous advantageous attributes of rabbits; which include low cost management requirements, rapid growth rate, short gestation and generational interval, prolificacy, genetic diversity and the ability to utilize forage and agricultural by-products; they have been adjudged to be a veritable option in solving the animal protein needs of developing countries and provide sustainable income for the poor rural dwellers [2].

Unfortunately, rabbit production in developing countries like Nigeria has been described as rudimentary, developing or emergent; when compared with some developed countries [2]. Therefore, there is need to increase research geared towards enhancing rabbit production in developing countries, which will adhere to the call for organic farming [3], so as to enable subsistent rabbit farmers, currently dominating the rabbit production industry in developing countries to be familiar with the research findings and employ same with ease.

In recent times, numerous plants and plant products have been employed in the production of several animal species; *Moringa oleifera,* which is the most widely distributed species of *Moringaceae* family, with high nutritional values, has been reported to possess numerous beneficial properties leading to impressive range of medicinal uses throughout the world [4]. Recently, Moringa has been widely used in various researches involving animal models, including rabbits; as a feed resource or to influence organ tissues or physiological activities in the animals' body [5]. Moringa leaf powder has been reported to reverse the decreased testis and epididymal weights in hyperglycaemic

mice and also increased sperm count and motility even as it decreased sperm mortality [6]. At 15 % inclusion level in the diet of rabbit bucks, *Moringa oleifera* leaf meal had no adverse effect on the testicular morphometry and epididymal sperm quality of rabbit bucks [7]. *Moringa oleifera* has also been successfully used to replace *Centrsoma* pubescens in the diet of rabbits without any untoward effect on their reproductive performance [8]. Based on numerous documented beneficial effects of *Moringa oleifera* in livestock production, this research was carefully designed to assess the reproductive, haematological and serum biochemical influences of aqueous extracts of *Moringa oleifera* leaves on adult New Zealand rabbit bucks.

Materials and Methods

This research lasted for 4 weeks and was carried out at the Rabbitry Unit, Teaching and Research Farm, Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo State. Imo State is situated in South Eastern agro-ecological zone of Nigeria, and lies between latitude $4^{o}4^{1}$ and $6^{o}3^{1}$N, and longitude $6^{o}15^{1}$ and $8^{o}15^{1}$E. Owerri is about 100 m above sea level with mean annual rainfall of 2500 mm, temperature range of 26.5 – 27.5 ^{o}C and humidity range of 70 – 80 %. Dry season duration (months with less than 65 mm rainfall) is three months, which takes place during the months of December, January and February [9].

Eighteen adult New Zealand rabbit bucks of average age of 7.4 months were purchased from a reputable farm in Umudike, Umuahia, Abia State and used in this study. On weight equalization basis, the rabbits were divided into three groups of 6 rabbits each, randomly assigned to three treatments (MT_0, MT_{100} and MT_{200}) and replicated three times to contain 2 rabbits per replicate. They were housed separately in 2 hutches of 12 cages each, while standard management practices as described by [1] were followed throughout the experiment.

Fresh leaves of *Moringa oleifera* were harvested at Michael Opkara University of Agriculture, Umudike and Federal University of Technology, Owerri. The leaves were air-dried, ground into leave meal and stored in a moisture-proof container; and used when needed. Aqueous extracts of *Moringa oleifera* leaves, which composed the treatments, were then prepared from the leave meal. Treatment two and three (MT_{100} and MT_{200}) were prepared by dissolving the Moringa leave meal in boiled distilled water at the rate of 100 g/L and 200 g/L, respectively. The solutions were allowed to cool and then filtered with a clean cotton cloth. The treatments were administered to the animals as drinking water throughout the experimental period. Distilled water without aqueous extracts of Moringa leaves were administered to the animals as a control treatment (MT_0) and served as treatment one. The rabbits were fed a standard rabbit diet (Crude protein = 14 %; Crude fibre = 15 %; Crude fat = 3 %; Digestible energy = 2,200 Kcal/kg) without forage supplementation.

After two weeks of treatment administration, semen samples were collected twice a week (Mondays and Thursdays) for two weeks, using an artificial vagina (AV) described by [10]. The semen analysis was carried out according to procedures outlined by [11]. Following the introduction of a teaser rabbit doe into the cage of the rabbit buck and with the aid of a stop watch, reaction time (secs) was determined by calculating the time it took the rabbit buck to mount the rabbit doe, while libido score was determined by recording how many times the buck tries to mount the doe per minute.

Blood samples were collected once a week in the 3rd and 4th weeks of treatment administration through the marginal ear vein of the rabbits, using needle and syringe. Blood samples for haematological analysis were discharged into Ethylene Diamine Tetra-acetic Acid (EDTA) bottles, while blood samples for serum biochemical analysis were discharged into plain bottles (without EDTA). The haematological and serum biochemical analyses were carried out following standard procedures as outlined by [12]. Samples and data were collected from three rabbits per treatment; that is, one per replicate.

Data generated from this study were subjected to one way analysis of variance using the General Linear Model (GLM) of the Statistical Analysis System [13]. Significantly ($P<0.05$) different means were separated using Duncan's Multiple Range Test, of the same software.

Table 1: Reproductive performance of adult rabbit bucks administered aqueous extracts of *Moringa oleifera* leaves

Parameters	MT_0	MT_{100}	MT_{200}	SEM
Semen volume (ml)	0.82^a	0.71^b	0.68^b	0.03
Total sperm count (x 10^6)	96.32^b	110.50^a	112.66^a	3.27
Sperm concentration (x 10^6/ml)	119.34^b	158.12^a	166.67^a	8.34
Individual sperm motility (%)	70.20^b	76.41^a	78.40^a	2.01
Sperm mass motility (0-4)	2.81^b	3.20^a	3.21^a	0.12
Live sperm (%)	80.02^b	86.46^a	88.32^a	2.11
Abnormal sperm (%)	14.20^a	10.46^b	8.50^c	0.62
Reaction time (Secs)	8.45^a	5.86^b	5.41^b	0.81
Libido score (mounts/minute)	7.26^c	9.43^b	11.40^a	0.64

^{abc}: Means within row with different superscripts are significantly (P<0.05) different.

Table 2: Haematological parameters of adult rabbit bucks administered aqueous extracts of *Moringa oleifera* leaves

Parameters	MT_0	MT_{100}	MT_{200}	SEM
Packed cell volume (%)	38.20^b	39.78^b	42.80^a	1.00
Haemoglobin concentration (g/dl)	13.00^b	13.27^b	14.10^a	0.26
Red blood cells (x 10^6/ml)	5.22	5.18	5.28	0.04
Total White blood cells (x 10^3/ml)	8.73^b	9.81^a	9.92^a	0.33
Lymphocytes (%)	58.50^b	62.00^{ab}	64.66^a	2.01
Heterophils (%)	39.50^a	36.50^{ab}	33.33^b	2.01
Monocytes (%)	2.00	1.50	2.00	0.17

^{ab}: Means within row with different superscripts are significantly (P<0.05) different.

Table 3: Serum biochemical parameters of adult rabbit bucks administered aqueous extracts of *Moringa oleifera* leaves

Parameters	MT_0	MT_{100}	MT_{200}	SEM
Total protein (g/dl)	5.10^b	5.84^a	6.02^a	0.20
Albumin (g/dl)	3.50	3.56	3.60	0.40
Globulin (g/dl)	1.60^b	2.28^a	2.42^a	0.19
Urea (mg/dl)	18.86^b	19.04^b	24.47^a	1.62
Creatinine (mg/dl)	1.64	1.63	1.68	0.04
Urea/creatinine ratio	11.60^b	11.68^b	14.52^a	0.91
Glucose (mg/dl)	110.44^a	96.47^b	87.38^c	2.89
Cholesterol (mg/dl)	96.71^a	75.22^b	63.91^c	2.91
Alkaline phosphatase (IU/L)	36.78	35.55	35.60	0.99
Alanine transaminase (IU/L)	72.51	72.47	73.53	0.82
Aspartate transaminase (IU/L)	98.43	98.98	98.46	0.43

^{abc}: Means within row with different superscripts are significantly (P<0.05) different.

Results

The results of the reproductive performance of rabbits administered aqueous extracts of *Moringa oleifera* leaves are presented in Table 1. Semen volume of the rabbits treated with the extracts (MT_{100} = 0.71 ml; MT_{200} = 0.68 ml) were similar (P>0.05), but significantly (P<0.05) lower than semen volume of rabbits on the control (MT_0 = 0.82 ml). Total sperm count (x 10^6), sperm concentration (x 10^6/ml), individual sperm motility (%), sperm mass motility (0-4) and live sperm proportion (%) of the experimental rabbits recorded similar (P>0.05) values in MT_{100} and MT_{200} which were significantly (P<0.05) higher than values recorded for rabbits in MT_0. Abnormal sperm proportion (%) were significantly (P<0.05) higher in MT_0 rabbits (14.20 %) than in MT_{100} rabbits (10.46 %), which was significantly (P<0.05) higher than MT_{200} rabbits (8.50 %). Reaction time (secs) of MT_0

rabbits (8.45) were significantly (P<0.05) higher than reaction time of MT_{100} (5.86) and MT_{200} (5.41) rabbits, which were similar (P>0.05). Libido score of the experimental rabbits were 7.26, 9.43 and 11.40 mounts/minute for MT_0, MT_{100} and MT_{200}, respectively; and MT_{200} was significantly (P<0.05) higher than MT_{100} which was significantly (P<0.05) higher than MT_0.

Haematological analysis of the experimental rabbits (Table 2) recorded significantly (P<0.05) higher values in packed cell volume (PCV) and haemoglobin (Hb) concentration for rabbits on MT_{200} (42.80 %; 14.10 g/dl) than for rabbits on MT_0 (38.20 %; 13.00 g/dl) and MT_{100} (39.78 %; 13.27 g/dl) which were similar (P>0.05). Total white blood cells (tWBCs) were similar (P>0.05) between rabbits on MT_{100} (9.81 x 10^3/ml) and MT_{200} (9.92 x 10^3/ml), which were significantly (P<0.05) higher than tWBCs of rabbits on MT_0 (8.73 x 10^3/ml). The percentage lymphocyte count of the rabbits on MT_{200} (64.66 %) were similar to that of MT_{100} rabbits (62.00 %), but significantly (P<0.05) higher than lymphocyte count of MT_0 rabbits (58.50 %), which were similar to MT_{100}. Percentage heterophil count of the rabbits on MT_0 (39.50 %) were similar to percentage heterophil count of MT_{100} rabbits (36.50 %), but significantly (P<0.05) higher than percentage heterophil count of MT_{200} rabbits (33.33 %) which was similar to MT_{100}.

Total protein and globulin values recorded in the serum biochemical analysis of the rabbits (Table 3) were similar (P>0.05) in MT_{100} (5.84 g/dl; 2.28 g/dl) and MT_{200} (6.02 g/dl; 2.42 g/dl) rabbits, which were significantly (P<0.05) higher than total protein and globulin values recorded for MT_0 (5.10 g/dl; 1.60 g/dl) rabbits. Serum urea content and urea/creatinine ratio of the experimental rabbits were significantly (P<0.05) higher in MT_{200} (24.47 mg/dl and 14.52) rabbits than in MT_0 (18.86 mg/dl and 11.60) and MT_{100} (19.04 mg/dl and 11.68) rabbits, which were similar (P>0.05). Serum glucose and cholesterol of the rabbits were significantly (P<0.05) higher in MT_0 (110.44 mg/dl and 96.71 mg/dl) rabbits than in MT_{100} (96.47 mg/dl and 75.22 mg/dl) and MT_{200} (87.38 mg/dl and 63.91 mg/dl) rabbits; and MT_{100} significantly (P<0.05) higher than MT_{200}.

Discussion

The result of the reproductive analysis of the rabbit bucks administered aqueous extracts of *Moringa oleifera* leaves which recorded significant decrease in the semen volume of the rabbits could not be due to reduction in sperm production, but definitely due to reductions in seminal fluid production by the accessory sex glands; since total sperm count and sperm concentration were significantly higher in the treated rabbits. However, the mechanism and the accessory sex glands affected could not be ascertained in this experiment. The significant increase in the total sperm count of the treated rabbit bucks corroborates earlier reports that extracts of *Moringa oleifera* leaves supports and enhances spermatozoa production (spermatogenesis) [14]. This could be partly due to the great antioxidant and nutritional potentials of moringa leaves [15, 16]. Aqueous extracts of *Moringa oleifera* leaves also significantly increased spermatozoa motility and percent live sperm, while percent abnormal sperm was significantly reduced in the treated rabbits in a dose dependent manner, thereby demonstrating a role for aqueous extracts of *Moringa oleifera* leaves in spermatozoa nourishment. Sexual effects of *Moringa oleifera* leave extracts in the New Zealand rabbit bucks were demonstrated by the significant reduction in reaction time (secs) and a significant increase in the libido score (mounts/minute); in a dose dependent manner. Similar results were obtained in sexual activity of male albino rats treated with aqueous extracts of *Moringa oleifera* seeds [17]. This could be attributed to increased serum testosterone levels of the rabbit bucks, following administration of the aqueous extracts of *Moring oleifera* leaves; since increased sexual activity or androgenic effects are linked with testosterone levels in the blood [18, 17].

Aqueous extracts of *Moringa oleifera* leaves at 200 mg administration caused a significant increase in the percentage packed cell volume (PCV) and haemoglobin (Hb) concentration of the rabbit bucks, thereby increasing the oxygen carrying capacity of their blood [19, 20]. The non significant difference in the red blood cell (RBC) counts of the experimental rabbits, under significantly different PCV values, probably suggests that the aqueous extracts of *Moringa oleifera* leaves mediated increase in sizes (hyperplasia) of the red blood cells without increase in their number. The total white blood cell (WBC) counts, although comprises an insignificant portion of the PCV, may have acted in an additive manner with the RBCs to contribute to the significantly higher value of PCV recorded in MT_{200} rabbits. The significantly higher values in total white blood cells recorded in the treated rabbits in the absence of a pathological condition, corroborates earlier reports that *Moringa oleifera*

leaves extracts mediates stimulatory effect on the WBC counts of Wistar albino rats [21], thereby increasing their cellular immunity, which has been reported as one of the attributes of *Moringa oleifera* [22, 23, 21]. From the result of the differential counts of the white blood cells, the lymphocytes and heterophils were predominantly seen. Obviously, the significant increase in the total white blood cell counts of the treated rabbits were as a result a corresponding increase in the lymphocyte count of the treated rabbits, which became significant at 200 mg administration of the extracts. The heterophil counts decreased in the extract treated rabbit bucks and became significant at 200 mg level of administration; which can be attributed to the antioxidant activity of the *Moringa oleifera* leave extracts [16]. This inference is sequel to the report that antioxidant activity decreases heterophil counts in the absence of an inflammatory condition [24, 20].

Administration of aqueous extracts of *Moringa oleifera* leaves significantly increased serum total protein (TP) in the treated rabbit bucks, probably due to the high amino acid content of the moringa leaves which has been used in several nutritional/feeding trials and reported to be a good source of protein for livestock [15, 5, 25]. It could also be that the aqueous extracts of *Moringa oleifera* leaves enhanced the mechanism for the synthesis of serum protein from available amino acids. The immunity role of *Moringa oleifera* leaves [22, 23, 21] is also demonstrated in the fact that the significant increase in the TP of the treated rabbits is actually as a result of the significant increase recorded in the serum globulin (component of total protein) of the treated rabbits. Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism, the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is synthesized to urea in the liver [19]. The increase in serum urea content of the treated rabbits, which became significant at 200 mg administration of *Moringa oleifera* leaves extracts, is not surprising due to the corresponding increase in the serum TP of the rabbits. Furthermore, the significant increase in the serum urea content of MT_{200} rabbits do not suggest any physiological failure nor portend danger for the animals, since it is within the normal range for rabbits; and the serum urea/creatinine ratio, which could serve as an indication of the functional status of the kidney were also within normal ratios [26]. Serum glucose and cholesterol values of the rabbits in this study corroborates earlier studies that reported significant reductions in the serum glucose and cholesterol of various animal species treated with various parts of *Moringa oleifera*[27, 28, 29]. Serum glucose and cholesterol which were significantly lower, in a dose dependent manner, in the rabbits receiving 100 and 200 mg aqueous extracts of *Moringa oleifera* leaves were definitely due to the anti-diabetic and anti-cholesterolemic activities of moringa leaves, which has been widely documented [27, 6, 28, 29].

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